Chapter V

Histological, Histopathological and Histochemical Observation and Discussion on Liver of Channa striatus (Bloch.) Exposed to Various Concentrations of Phosphate

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Liver :

Introduction and Review of Literature :

In general liver is a yellowish brown gland consisting of two main lobes that are sub-divided into smaller lobes. The lobes of liver extend on each side between skin and muscles. The gall bladder is a narrow sac between the intestine and the right lobe of the liver. Bile duct is a short tube opening into the intestine (Khanna, 1973). The histology of liver in fishes have been described by several investigators. Notable investigators who studied liver or digestive gland are Sarbahi (1940) on L. rohita, Kapoor (1953) and Sinha (1958) on Wallago attu, Weisel (1973) on P. spathula. Even some ultrastructural studies are also made to find out minor details of the histology of river by Scarpelli et. al., (1968), Vernier (1975).

Generalized studies of fish liver has been described by Khanna (1973). Accordingly to him the liver is composed of a large number of polyhedral hepatic cells, each of which contains granular cytoplasm and a central nucleus. Numerous bile ductules and blood capillaries are scattered in it.

Most of the studies on liver toxicity are carried out by using different pesticides to study histomorphological alterations in the hepatic tissue of fishes (King, 1962, Andrews et. al., 1966). Hinton et. al. 1973, Mukherjee and

Bhattacharya (1975). Relation between endrin concentration in water and histopathological changes in hepatopancrease amylase activity in C. bactrachus along with abnormal behaviour had been studied by Bhattacharya et. al. (1975). Degenerative changes including liver cord disarray, vacuolations, necrosis and inhibition of α amylase in C. punctatus was recorded by Shastry and Sharma, (1978). Chronic treatment of Thiodan E. C. 39 and Agallol '3' toxicity on liver of G. terntzi showed the destruction of hepatocytes, hyperplasia and hypertrophy of the of the islet cells (Amminikutty and Rege, 1977). Liver necrosis or atrophy occurred in the central and peripheral zones due to aldrin toxicity in C. carpio (Ratnakar and Awasthy, 1979). Malathion induced lesion in the liver of C. punctatus was noticed by Dubale and Shah (1979), whereas sumithion induced changes were observed in C. batruchus (Mandal and Kulshrestha, 1980). Squin (Quinalphos) toxicity was studied in liver of two cyprinid fishes B. ticto and Rasbora daniconis (Shareef et. al., 1986). Recently histopathological study of carp Labeo rohita exposed to hexachlorocyclohexane has been carried out by Das and Mukharjee (2000) showed various histopathological alterations in the liver showing mild congestion of blood vessels and marked swelling of the hepatocytes in places with areas of diffuse necrosis.

Phenol and their derivatives were tested by few investigators. The effects of pure phenol were studied on the liver of H. fossilis by Chatterjee et. al., (1983). Whereas phenol, 2, 4 dinitrophenol and pentachlorophenol and their combinations have been used to observe their effects on histopathology of N. notopterus (Gupta and Dalela, 1986). The effect of polychlorinated biphenyl in liver of S. gairdneri has been studied by Hacking et. al., (1978) at the ultrastructural level.

Alkaline phosphatase, glucose 6 phosphatase, ATPase and lactic dehydrogenase (LDH) were found to be decreased due to phosphamid toxicity in liver of N. denisonii (Rashatwar and Ilyes, 1984). Nuclear glycogen in hepatocytes in liver of R. ocellatus and its changes during development were reported by Thiyagarajah and Grizzle (1986). Mukherjee and Bhattacharya (1975) have observed disarry necrosis and disintegration of liver in O. punctatus and C. batrachus exposed to various industrial pollutants. Tripathi et. al., (1974) have studied the anatomical changes by urea toxicity in L. rohita. Sriwastawa and Srivastava (1985) reported the urea induced histopathological changes in the liver of C. punctatus.

Besides, pesticide effects, the histopathological changes in the liver due to heavy metal toxicity have been reported in number of fish species. The histopathological changes in the liver due to copper toxicity in C. punctatus have bee reported by Gupta and Rajbanshi (1986) and due to copper and Zinc in P. conchorius (Kumar and Pant, 1981) and due to copper sulphate in C. fasciata (Singh, 1983). Gill and Pant, 1981 investigated liver in order to understand biochemical and hematological responses to mercury toxicity in P. conchonius. Residual mercury in liver of fishes from estuary near costic chlorine industry was estimated by Shaw et. al., (1985). Cadmium toxicity in liver of fishes have been studied by Das and Banerjee (2000) for microsomal glucose 6 phophatase activity and serum glucose level. Shah (2002) studied the behavioural abnormalities of Cyprinon Watsoni on exposure to copper and zinc.

In the histochemical observations, PAS reactive glycogen in the liver of Catla fingerlings (Saxena, 1966) and in murrel fish O. punctatus (Shafi,

1977) have been noted. In sumithion toxicity fall in hepatic glycogen was significant (Koundinya and Ramamurthi, 1979).

From the review of the literature it seemed that there are almost no studies related to phosphate induced histopathological changes in the liver of fresh water fishes and there was not a single reference to show alterations in the mucosubstance in the hepatic tissue.

Therefore, a commonly found increased phosphate concentration due to sewage was selected for the present investigation to show its pathological effects on the histology and mucosubstance histochemistry in the liver of a fresh water fish Channa striatus.

1) Histological observation on the control fish liver :

histological observations of control C. striatus showed a continuous mass of hepatic cells forming hepatic cords (Plat No. 2, Fig. 1). The hepatic cells were large, hexagonal with centrally placed nucleus and homogenous cytoplasm. The formation of hepatic lobules of hepatic cells was less distinct. A large number of blood sinusoids appeared amongst the cords of hepatic cells (Plate No. 2, Fig. 1). Thin bile canaliculi were observed between the hepatic cells. Hepatic ducts stained darkly with PAS (Plate NO. 2, Fig. No. 1) Central venule of hepatic lobule appeared very distinct (Plate No. 2, Fig. 1).

2) Histopathological observations in the liver due to phosphate intoxication :

Histopathological alternation in the hepatic cells of the liver due to phosphate are photomicrographically illustrated in (Plate No. 2, Fig. 2 to 8). The histopathological changes are as follows –

PLATE NO. 2

Histology, histopathology and histochemistry of Liver of control and experimental fish C. striatus(Bloch.)

Fig. 1 : T.S. of liver of control fish stained with H - E X 1200.

Fig. 2 : T.S. of liver of fish exposed to 0.015 M phosphate for 48 hrs. stained with H. E. X 1200.

Fig. 3 : T.S. of liver of control fish stained with PAS X 280.

Fig. 4 : T.S. of liver exposed to 0.01 M phosphate for 24 hrs. stained with PAS X 330.

Fig. 5 : T.S. of liver of control fish stained with AB pH 2.5 – PAS X 240.

Fig. 6 : T.S. of liver of fish exposed to 0.01 M phosphate stained with AB pH 2.5 – PAS X 1320

Fig. 7 : T.S. of liver of control fish stained with AB pH 1.00 – PAS X 1120.

Fig. 8 : T.S. of liver of fish exposed to 0.007 M phosphate stained with AB pH 1.00 – PAS X 1120.

Abbreviations :

CVL - Central Venule of Lobule

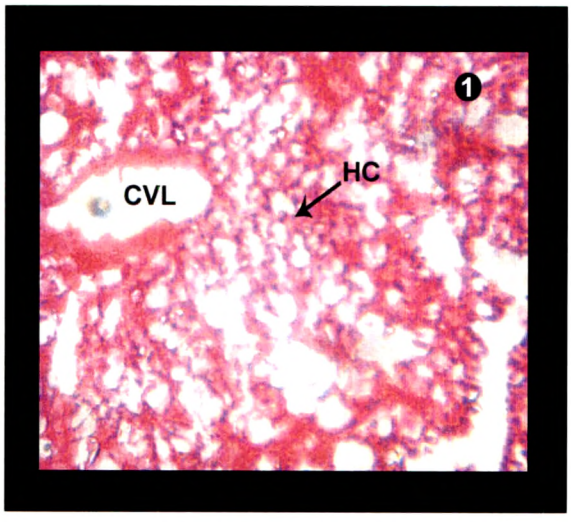
HC - Hepatic Cells

FN - Focal Necrosis

HL - Hepatic Lesion

HD - Hepatic Duct

PLATE NO. 2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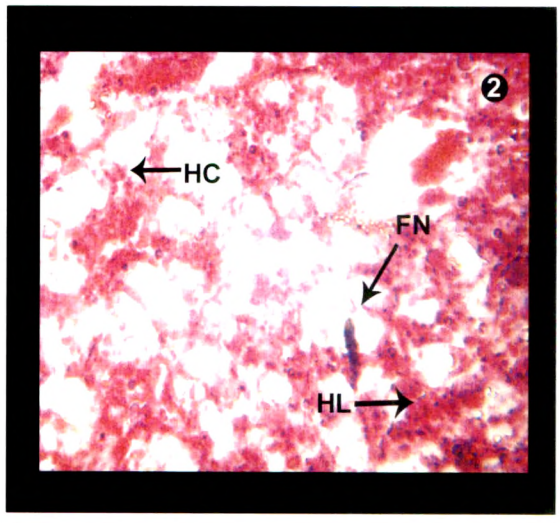
CVL
HC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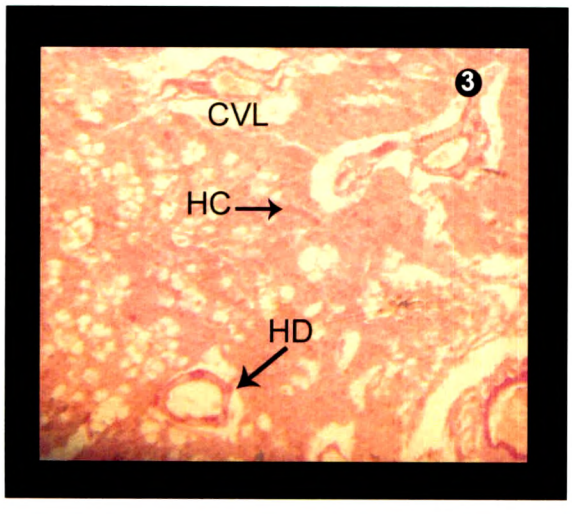
FN
HL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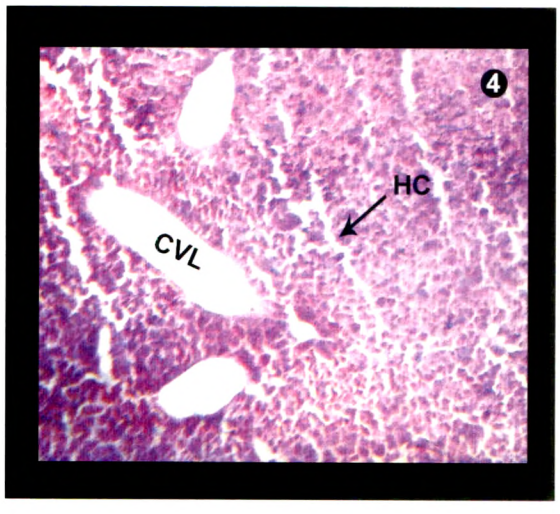
HC
HD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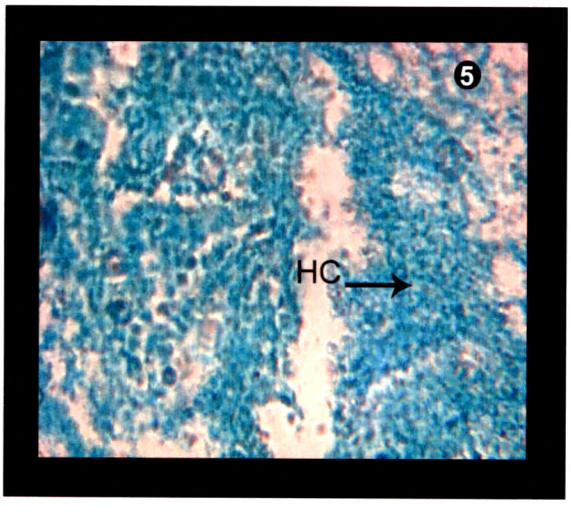
CVL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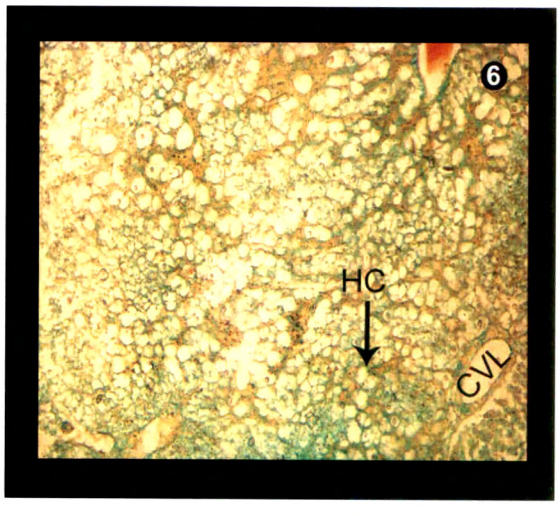
HC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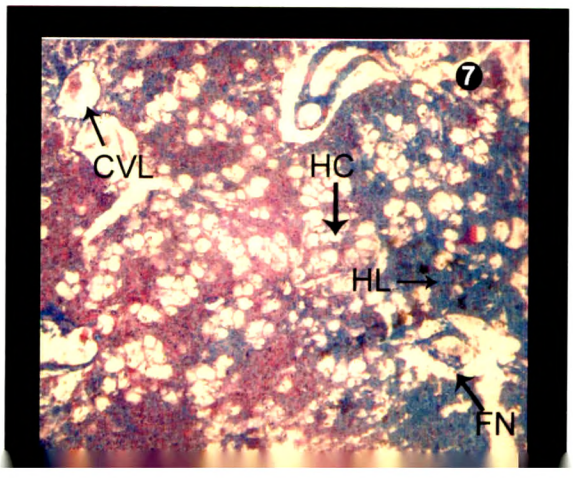
HC
CVL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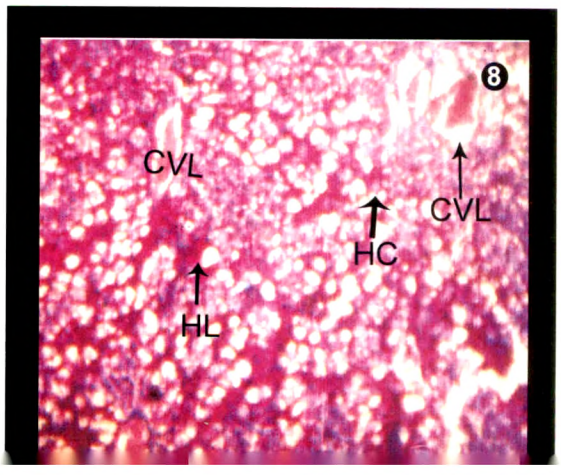
CVL
HC
HL
FN


8
CVL
CVL
HC
HL

0.006 M Phosphate :

Fish exposed to 0.006 M phosphate seemed to be not much affected. Liver showed almost regular continuous mass of hepatic cells. The hepatic cells were of normal size, hexagonal shape with centrally placed nucleus. Bile canaliculi, Central lobule of venule, hepatic ducts appeared almost normal. No distinct histopathological changes were observed in the liver.

0.007 M Phosphate :

Liver in fish exposed 0.007 M phosphate progressively showed partial disrupted architecture at 24 to 96 hrs. of exposure with diffused areas of necrosis. Central veins of the lobules showed partial distension. Hepatic cells showed slight increase in the size and vacuolation of the nuclei. Partial damage was evident especially in the tissue surrounding the sinusoids. The staining intensity of staining reactivity increased in the hepatic cells.

0.01 M Phosphate :

Liver in fish exposed to 0.01 M phosphate showed still further progression in the disruption of architecture at 24 to 96 hrs. Diffused areas of necrosis became more prominent. Sinusoid became more distended. Central lobular veins severaly damaged. Progressed vacuolization of the hepatic cells was predominant. Hypertrophy of the hepatic cells was also observed.

0.015 M Phosphate :

The severe degenerative changes were observed showing disarray, vacuolation and necrosis in hepatic cells (Plate No. 2, Fig. 2, 4, 6 and 8).

Focal necrosis and hepatic lesions were progressively and predominantly observed at 24 and 48 hrs. of exposure (Plate No. 2, Fig. 2).

Normal architecture of the liver was markedly disrupted. Central lobular venules appeared severely damaged due to swelling and degeneration of the endothelial cells. Vacuolar degeneration of cytoplasm, necrosis and hypertrophy was evident. Hepatic cells even ruptured in some areas were observed.

3) Histochemical observations on the control and exposed fish liver to various concentrations of phosphate :

The histochemical observations on some important staining reactions employed in the present investigation of the liver of C. striatus are recorded in Table No. 5.1 according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The distribution of mucosubstances in the hepatic cells and endothelial cells of central venule of lobule of the liver of control fish and intoxicated fishes are photomicrographically illustrated in Plate No. 2 fig. 2 to 8.

On the basis of elaboration of mucosubstances hepatic cells and endothelial cells of central hepatic venules showed responses and a brief summary of results from the various histochemical techniques are tabulated in Table No.

Control Fish :

Hepatic cells of C. striatus stained intensely with PAS (Plate No.2, Fig. 3) and Endothelial lining of CVL was intensely stained. Subsequently ABpH-1, ABpH-1-PAS, ABpH-2.5 AND ABpH-2.5-PAS reactivities in hepatic cells showed moderate staining to AB-1 and AB-2.5 where as AB-1PAS and AB-2.5-PAS showed moderate and intense staining respectively. Indicating hepatocytes elaborated glycogen and moderate amount of sulphated and carboxy mucins.

Endothelial cells also show intense PAS and moderate reactivities to other techniques.

Fish liver exposed to phosphate concentrations :

0.006 M Phosphate :

The PAS reactivity is slight decreased progressively from 24 hrs to 96 hrs. in hepatic cells. Endothelial cells showed progressive increase in sulpho and carboxy mucins.

0.007 M Phosphate :

The PAS reactivity of hepatic cells is still progressively decreased from 24 to 96 hrs. exposure and AB-2.5 showed slight increase indicating increase in secretion of carboxy mucins where as ABI-1 decreased indicating less sulpho mucins. AB-1-PAS and AB-2.5-PAS showed moderate reactivities. Endothelial cell shoed intense reactivities to PAS as well as AB-Ph-1 and AB-Ph-2.5 techniques showing increase in mucus secretion.

0.01 M Phosphate :

The PAS reactivity of hepatic cell is reduced progressively indicating depletion of glycogen in the liver. AB-pH-1 and AB- pH-2.5 reactivity still decreased.

In the endothelial cells of CV, the PAS, AB- pH-1, AB- pH-2.5 reactivities increased indicating more secretary activity in the endothelial cells. AB-1-PAS and AB-2.5-PAS reactivities are moderate.

0.015 M Phosphate :

PAS reactivity in the hepatic cells is meagre indicating depletion of glycogen content. AB-pH-1 and AB-pH-2.5 reactivities increased. The endothelial

cells of central venule of lobule showed intense staining with AB-pH-1 and loss of glycogen in liver tissue. AB-pH-2.5 indicating more mucus secretion.

Table No. 5.1

Histochemical observations on mucosubstances in the liver of freshwater fish Channa striatus (Bloch)

Sr. No.	Histochemical Techniques	Conc. of Phosphate	Generalized reactivities considering 24, 48, 72 and 96 hrs taken together	
			Hepatic Cells	CVL Endothelial cells
1	PAS	C	+++	++
		0.006 M	+++	++++P
		0.007 M	++	++P
		0.01 M	++B	++P
		0.015 M	+	++++P
2	AB pH 1	C	-	-
		0.006 M	++B	++B
		0.007 M	++B	++B
		0.01 M	+B	+++B
		0.015 M	+B	+++B
3	AB pH 1 - PAS	C	-	-
		0.006 M	++PB	+++
		0.007 M	++PB	+++
		0.01 M	+++PB	++
		0.015 M	+++PB	+
4	AB pH 2.5	C	-	-
		0.006 M	+++B	++
		0.007 M	+++B	++
		0.01 M	++	+++
		0.015 M	++	++++
5	AB pH 2.5 - PAS	C	-	-
		0.006 M	+++PB	++
		0.007 M	+++	+++
		0.01 M	++	++
		0.015 M	++PB	+++

Discussion :

The liver is the primary organ for detoxication of toxicants. Wide varieties of insecticides and several toxins tend to accumulate in high concentrations within it (Metelev, et. al., 1971) and the liver gets damaged. Hinsen et. al., (1971) studied fish exposed to pesticides under laboratory conditions and observed that liver contained highest pesticides concentration. Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pycnosis of nuclei and of Heteropneustes fossilis while subjecting them to acute thiodan toxicity. Similar changes were recorded in our present study with phosphates at higher concentration. Histopathological lesions in the hepatopancreas of many fishes have also been reported in relation to pesticide toxicity (Bhattacharya, et. al., 1975, Shareef et. al., 1986)

Mobilization of liver glycogen is also reported in many studies (Bhattacharya et. al., 1975), Begum, 1987). Destrophy of hepatic parenchyma and sedimentation of yellow haemoglobinogenic pigment in the liver of carps exposed to phenol was also reported (Bhattacharya, et. al., 1975).

Present study provides the information of effects of phosphate on the liver of C. striatus.

i) Histology :

Histological architecture of control fish C. striatus showed similarity with other carnivorous fish species. All the usual features such as hepatic ducts and dispersed pancreatic tissue was also observed in C. striatus. Similar type of histological features were observed by Bhattacharya et. al., 1975 in C. batrachus and Mukharjee and Bhattacharya, 1975.

ii) Histopathology :

The phosphate more than 2 mg/l in open water gives a sign of organic pollution (Pomeroy, et. al., 1965). A histopathological study of carp (Labeo rohita) exposed to hexachlorocycloxane indicated marked swelling of the hepatocytes in places with areas of diffuse necrosis (Das and Mukherjee, 2000). The phenolic compounds entered in the blood circulation damage liver first. Splitting, swelling liquefaction, hypertrophy, necrosis and vacuolation have been observed by Mitrovic et. al., (1968), Matie (1976) and Gupta and Dalela ((1986) after sublethal treatment with phenolic compounds.

In the present investigation phosphate toxicity showed histopathological alterations in the liver of C. striatus. The normal architecture of liver tissue was markedly disrupted especially to higher concentrations 0.01 M and 0.015 M phosphate. Marked swelling of the hepatic cells in places with areas of diffuse necrosis. Sinusoids in most cases were distended and central venule of lobule appeared severely damaged due to marked swelling and degeneration of endothelial living cells .

Histopathological changes in fish treated with cadmium chloride include liver cell damage, vacuolar degeneration of cytoplasm necrosis and hypertrophy. (Srivastava and Srivastava, 2007), Bhatacharya et. al., (1975) also reported repture and and vacuolation of liver cells of Clarias batrachus following exposure to 0.005 ppm endrin. DDT and related compounds have been reported to produce similar histopathological changes in several teleostean species (Mathur, 1972), Khan (2006) also reported similar changes in the liver of guppy, Lebistes reticulates after $CdCl_2$ treatment. Intoxication with endrin to teleost fish Channa

punctatus was characterized by hypertrophy, necrosis and vacuolation of hepatic cells and cirrhosis (Shastry and Sharma, 1978).

In the present study also similar changes were observed. The fish adjust itself to cope up with extrametobolic laod by compensatory hypertrophy of the liver cells i.e. increase in size of hepatic cells and hypertrophy of nuclei and vacuolized cytoplasm. This is probably due to depletion of glycogen and infiltration of lipids in the liver. Thus cytoplasm is migrated to periphery and the cells looked empty and large.

Histochemistry :

Shafi, 1977 studied the pattern of localization of glycogen deposits in the hepatocytes of O. punctatus. The similarity in the glycogen deposits in the liver of C. orientalis (P. S. Mane, 1988), and C. striatus in the present investigation seems to be due to the same piscivorous feeding habit. Alteration in the carbohydrate metabolites under stressful situations has been reported in fishes. Glycogen variations in the liver tissues, in different environmental conditions like change in salinity in C. punctatus (Bhatt et. al., 1979), following aerial exposure to cold stress in C. gachua (Khillare, 1986) have been reported. Qayyam and Shaffi (1977) reported that the mercury has different effects on glycogen content of liver of fish. At lower concentrations of Hg, glycogen content in the liver elevated and the higher concentration is decreased.

The results of present investigation conclusively proved that carbohydrate reserves were severely affected and depleted during exposure to higher concentration of phosphate in the liver of C. striatus. PAS reactivities indicate depletion of glycogen progressively in the liver of fish C. striatus exposed to higher concentration of phosphate.

Carboxy mucins and sulphomucin reactivities indicate that even though these mucins are not normally synthesized and deposited in the liver they might have been from other sources. Exposure to progressively higher concentration of phosphate increased the depositions of these mucosubstances might be a protective mechanism.